

Johannes Lindh

**STUDIES ON SOME ASPECTS OF
EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS**

Lund 1977

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Experimental Allergic Encephalomyelitis

A K A D E M I S K A V H A N D L I N G

som med vederbörligt tillstånd av Medicinska fakulteten i Lund
för avläggande av medicine doktorsexamen kommer att offentligen
försvaras i stora föreläsningssalen å Anatomiska institutionen,
Biskopsgatan 7 i Lund fredagen den 18 februari 1977 kl. 09.00,

av

Johannes Lindh

Läkare, Klm.

Lund 1977

From the Tornblad Institute, University of Lund,
Lund, Sweden

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INTRODUCTION

Neurological complications sometimes followed repeated vaccination of humans against rabies with a mixture of attenuated virus and spinal cord of rabbit. This led to the study of an autoimmune experimental disease of the central nervous system (CNS): experimental allergic/autoimmune encephalomyelitis (EAE). This disease can be produced with a single injection of antigen (CNS-tissue or different preparations of it) if emulsified in Freund's complete adjuvant (FCA). Essentials in this adjuvant are the mycobacteria of any strain and the oil, giving the emulsion. Other adjuvants described are pertussis vaccin and carbonyl iron.

Adams (1959) suggested that some human demyelinating diseases - acute necrotizing haemorrhagic leukoencephalitis, acute disseminated encephalomyelitis, multiple sclerosis (MS), and Schilder's disease - have an autoimmune component in the aetiology. During recent decades, EAE has been used as a model, especially for MS. Although differences exist, there are many features in common (cf. reviews by Nilsson 1972, Mackay et al. 1973, Paterson 1973). This justifies comparative aspects on EAE and MS.

In EAE, the aetiological agent is an antigen isolated from CNS myelin. It is a basic protein, also called encephalitogenic protein (EP). The bovine EP is well characterized. It is built up of 169 amino acids and has a molecular weight between 18,000 and 19,000. Minor differences between EPs of different species origin exist in the amino acid sequence. Two EPs have been derived from rat myelin, the smaller being analogous to the larger except for a deletion of 40 amino acids within the C-terminal part. A region of an EP that comprises a number of amino acids capable of inducing EAE is called an encephalitogenic site. Different species do not always respond to the same encephalitogenic site(s). (Bergstrand 1977 fully reviews this.) It can be briefly exemplified by the guinea pig, where the major encephalitogenic site is around the single tryptophan residue (residue 115; numbering according to Brostoff et al. 1974), and the rat which has the major encephalitogenic site within the sequence 43-88.

The aetiology of MS is unknown. At present, the possibility of a virus aetiology is much debated. Many different viruses have been pointed out. Antibodies to measles virus appear in higher titres and frequencies among MS-patients than among controls; this has been studied by several groups, e.g. Vandvik & Degré (1975). There are also diseases of the CNS where a virus with a prolonged incubation or latency time, so-called "slow-virus", is

the aetiological agent, e.g., subacute sclerosing pan-encephalitis (SSPE), Kuru, and Creutzfeldt-Jacobs disease. In SSPE, an immunological abnormal reaction against a measles-virus infection several years before is believed to be the explanation of the disease.

The pathology of EAE in guinea pigs is briefly described (Waksman & Adams 1962, review by Rowe 1968). Clinical symptoms usually appear 10-15 days after immunization, and might begin with weight loss. The animals move slowly and reluctantly. This is followed by rapid ascending paralysis - hindlimbs, back, forelimbs - which is first flaccid but can become spastic. Urine and faecal incontinence are included in the picture, which can be complicated by pyelitis. Some animals die within two weeks of the first symptoms. Survivors often go to full reconstitution. Histological lesions, usually appearing in the CNS several days before the onset of clinical abnormalities, start with perivenous infiltration by mononuclear cells. These invade the nervous parenchyma and seem to destroy myelin directly. Plasma cells begin to appear 2-3 days later in the vascular cuffs, choroid plexuses, and meninges. There are marked species differences in the histological picture. Ferraro & Roizin (1954) found a striking histological resemblance between MS and EAE in monkey but not in guinea pig. Demyelination, which is typical of MS, can be minimum or absent at EAE in rats (Cavanagh 1956).

The disease course of EAE and MS differs. Characteristically, MS is a relapsing disease but EAE is monophasic. However, in recent years, several reports on relapsing EAE have appeared, favouring EAE as a model for MS. Thus McFarlin et al. (1974) and Paterson (1974) reported a form of recurrent EAE in the Lewis rat; we also saw this in our laboratory. Levine & Sowinski (1975) reported that adrenalectomy could induce relapses in the Lewis rat. In guinea pigs, a chronic form of EAE has been reported (Stone et al. 1969).

It is generally agreed that genetical factors are of importance in the development of EAE and MS. At EAE, the response of the immune apparatus at immunization is said to depend on immune-response genes (Ir-genes) closely linked to the major histocompatibility complex (MHC) (Gasser et al. 1973, 1975, Williams & Moore 1973). The incidence of MS is greater among relatives of MS-patients than among healthy individuals; this, of course, is compatible with an exogenic aetiological agent. For instance, studies on monozygotic twins indicate that exogenic factors are of importance, as one of such twins can develop MS and the other can remain healthy throughout life. Perhaps MS-patients possess certain Ir-genes that make them respond immunologically abnormally. Support exists for such a hypothesis where the responsible genes seem to be in linkage-disequilibrium with certain HLA-specificities.

It is generally accepted that cell-mediated immunity operates

in the pathogenesis of EAE. The cell-mediated immunity to EP can be studied both in vitro (e.g. lymphocyte transformation tests, cytotoxicity tests) and in vivo (delayed type skin hypersensitivity). But immunization brings about changes also in other parameters: circulating antibodies against EP and biologically active neurotoxic factors. Added to cultured neural tissue, these factors cause glia death, demyelination, or suppression of polysynaptic electrical activity (cf. reviews by Nilsson 1972, Mackay et al. 1973, Paterson 1973).

In MS, cell-mediated reactions to EP are described. Källén et al. (1977a) recently gave a literature review. Interestingly, such reactions can be seen also in other neurological diseases, such as amyotrophic lateral sclerosis or after cerebral infarction, or at carcinoma and even in apparently healthy individuals, but it is most prominent and frequent at MS (review by Nilsson 1972, Bergstrand et al. 1974, 1975, Berg et al. 1975). In MS, attempts to detect humoral antibodies against EP have usually been rather unsuccessful, as pointed out by Lisak et al. (1968). However, Caspary & Chambers (1970), using an immuno adherence technique, reported such antibodies in MS and also in other neurological diseases. On the other hand, neurotoxic factors, analogous to those at EAE have been frequently described (cf. reviews by Nilsson 1972, Mackay et al. 1973, Paterson 1973), and evidence has been obtained for an immune response to unidentified antigens in MS. In cerebrospinal fluid (CSF), an increased level of IgG (oligoclonal) can be seen, an abnormal kappa:lambda light chain ratio, and mononuclear cells. Laurell & Link (1972) reported the presence of complement-fixing antibody antibodies in CSF of MS patients, but not in control patients with other neurological diseases.

Thus there are differences between EAE and MS that make EAE a less suitable model for MS. EAE is a very "clean" immunological model, probably regulated only by genetical factors, whereas the human disease is an end-result of a highly complex set of interactions of both exogenic and genetical factors. None the less, EAE is a model which offers possibilities to study some immunological phenomena present at MS.

AIM OF THE PRESENT INVESTIGATION

The aim of this investigation was to examine certain aspects of EAE with the object of casting light on similar phenomena reported in MS by other authors and contributing to the understanding of some phenomena at EAE.

1. During recent years, abnormalities in the cell-mediated immune reaction called the mixed leukocyte reaction (MLR) at MS and in other diseases of presumed similar aetiology have been reported. This motivated a study to examine whether the same phenomenon was present in the animal model. Thus the MLR was studied in rabbits with EAE.
2. Encephalitogenic sites were well examined in the rabbit and relatively well in the rat. In the guinea pig, a dominating encephalitogenic site had been found, and it was of interest to study other parts of the EP for activity, and, if present, to quantify them.
3. As genetical factors seem to be important at MS, those factors were studied at EAE. However, hallmarks were obtained for a dominant gene for EAE-susceptibility (Gasser et al. 1973, Williams & Moore 1973) and for resistance (Perlík & Zídek 1974), whereas a later study by Gasser et al. (1975) indicated a much more complicated mode of inheritance. Therefore further studies in this field were motivated with strains of different susceptibility to EAE and also studies on the F₁ generation and backcross of F₁ generation with one of the parental strains.
4. As mentioned previously, there is an in vitro cell-mediated reactivity to EP at MS and also at EAE. The literature does not always report a correlation between EAE disease and in vitro tests in guinea pigs (cf. Webb et al. 1973) although another report does so (Lisak et al. 1975). In rats, a correlation seemed to exist (McFarlin et al. 1975a). A need for further studies on this aspect with rats of different genotypes motivated in vitro studies on cell-mediated immunity in the rats used for genetical studies.

The results are published in the following papers, which are the basis for the present thesis.

- I. Lindh, J. (1973) Mixed leukocyte reaction in rabbits with experimental autoimmune encephalomyelitis. Scand. J. Immunol. 2:417.

- II. Lindh, J. & Bergstrand, H. (1975) Disease-inducing activity of different parts of bovine encephalitogenic protein in guinea pigs. *Neurobiology* 5:137.
- III. Lindh, J. (1977) A quantitative study of encephalitogenic protein and peptides in guinea pigs. Accepted for publication in *Exp. Pathol.*
- IV. Lindh, J. (1977) Susceptibility to experimental allergic encephalomyelitis in two strains of rats and their hybrids. Accepted for publication in *Acta neuropath.*
- V. Lindh, J. (1977) Cell-mediated immune response to guinea pig and bovine basic proteins of myelin in Lewis and PVG rats and their hybrids. Accepted for publication in *Acta path. microbiol. scand. Sect. C.*
- VI. Lindh, J. (1977) Susceptibility to experimental allergic encephalomyelitis and cell-mediated immunity to myelin basic proteins and peptides in inbred Fischer rats.

Further original data are presented in Appendix I and II in this thesis. The above papers are referred to by their Roman numerals.

DISCUSSION

Mixed Leukocyte Reaction

In 1969, Astorga & Williams reported an altered reactivity in MLR of lymphocytes from patients with rheumatoid arthritis (RA). This was followed by similar studies in other diseases: MS, various arthritides, SLE, and scleroderma. Thus the MLR is often impaired between cells from patients with MS (Källén & Nilsson 1971, Cazullo & Smeraldi 1975), psoriasis arthropathica, pelvispondylitis, SLE or scleroderma (Hedberg et al. 1971). However, MLR is normal between cells from a patient with MS and a normal individual. The phenomenon is not disease-specific: cells from patients with MS react poorly with cells from patients with arthritis. A study of the effect of patient sera on a two-way MLR between normal individuals shows that a small depressive effect is present with the patient sera, but it is not of a magnitude that can explain the MLR impairment under study (Hedberg et al. 1973).

The MLR measures the blast-transformation and is quantified by uptake of added ^3H -methyl-thymidine. It is generally agreed that the T-cell is the reacting cell (cf. v.Boehmer 1974); it mainly reacts on lymphocyte defined (LD) antigens (also called MLR-antigens, and in homo, now designated HLA-D) on the cell-surface. The LD-antigens are determined by genes of the MHC and so too are the serologically defined (SD) antigens (in homo, now designated HLA-A,B, and C). It has been shown that among MS-patients there is an overrepresentation of the SD-antigens HLA-A3 and HLA-B7 (Jersild et al. 1972) and the LD-antigen HLA-DW2 (Jersild et al. 1973). There are different hypotheses about the implications of these findings in view of the impaired MLR. One possibility is that the greater similarities among MS-patients in HLA-D-coded antigens are responsible for the impaired MLR. This fits well with the normal MLR in patient-healthy combinations, but does not explain why cells from MS-RA combinations show impaired MLR. Another possibility is that there is an immunological defect in MS due to an allele in the I-region of the MHC. This gene should thus be closely linked to the HLA-loci and be in disequilibrium in the population with certain alleles of these loci. This could explain the impaired MLR also at MS-RA combinations, presuming a similar, but not identical, mechanism in RA. This is also compatible with the normal MLR in patient-healthy combinations, as it has been thought that the impairment in MLR could be due to a defect macrophage function and that normal macrophages from one donor would be sufficient for a full-blown MLR (Hedberg et al. 1971). A study on the MLR in rats challenged with EP obtained evidence for a macrophage defect (Axelsson & Källén 1976).

A study by Källén et al. (1975) demonstrated that the impairment of the MLR between a certain pair of MS-patients could remain constant when studied repeatedly for a considerable time: in those pairs, both patients often carried HLA-B7 antigens. Other pairs of MS-patients showed a significantly and markedly varying response to each other's cells in MLR when studied repeatedly over an extended time: then only one or neither of them carried HLA-B7 antigens. The impairment found in the former group was suggested to be related to the presence of DW2 antigens in both cell preparations. The variable impairment found in the latter group was thought to result from "immunosuppressive" factors not directly related to gene products.

Field et al. (1976) studied the MLR in MS by another technique: the macrophage electrophoretic mobility test, and they confirmed the impaired MLR in MS. However, another detail was reported: some clinically healthy children of MS families also showed an impaired MLR. Various interpretations of this can be discussed: i) identity in HLA-D, which is more likely to occur between relatives than others; ii) presence of an immunological abnormality in the healthy individual who has not yet precipitated an attack of, for instance, MS; iii) a postulated aetiological agent for MS has been presented to the healthy relative who is slightly immunologically abnormal, in a way resembling an animal challenged for EAE but not developing it.

Recently, Källén et al. (1976) pointed out and discussed problems that exist in DW2-typing of human leukocytes. As previous studies on HLA-D frequencies in MS do not always agree, the authors studied the problem of DW2-typing under these circumstances. The data indicate the possible existence of a confounding factor when cells from patients with MS are studied. This emphasizes need for care in the interpretation of such data.

In this context, it should be noted that a close association has been described between MS and the presence of another gene, Ag 7a, which gives a B-cell alloantigen (Winchester et al. 1975). Similarly, Terasaki et al. (1976), described an increased frequency of one B-lymphocyte antigen at MS, whereas five other specificities were found at a decreased frequency.

The impaired MLR at MS motivated a similar study on the model EAE. Paper I reports that the MLR is impaired in rabbits with EAE. This was later confirmed in studies on rats, challenged with EP in FCA, but not developing EAE (Axelsson 1975, Axelsson & Källén 1976). However, the rabbit cells showed an impaired MLR also with "healthy" cells, especially when the former were used as stimulator cells. This argues that the lymphocytes are changed in a way that masks the stimulating antigenic structures on their surfaces. One possible explanation of this would be the presence of a serum factor which could "cover" the lymphocytes in a way similar to that ob-

tained with an antilymphocytic antiserum, but other possibilities exist. Actually, in RA, there is evidence for a change in surface structure as one cause of the impaired MLR (Williams et al. 1971). However, the animal model differs from the human counterpart, as patient-healthy combinations show normal MLRs.

Paper I also reported that EAE-sera could depress a two-way MLR between cells of two healthy rabbits. This was further confirmed in a later study (Appendix I) which also demonstrated that the responsible factor(s) are labile and difficult to isolate and characterize. This labile character resembles that of the lymphotoxic activity described in MS-sera (Stjernholm et al. 1970, White et al. 1975). On the other hand, it argues against the possibility that it can be an antibody of the "protective" type described by Paterson et al. (1965) or an antilymphocytic antibody, as such antibodies would reasonably be easier to isolate. The MLR depressive effect of EAE-sera is analogous to that of MS-sera, even if it is weaker in the latter group (Hedberg et al. 1973). It is an open question whether it can be related to the serum-alpha-globulins, as has been done with the immunosuppressive activity of EAE-sera (tested in another system) by Vandenbark et al. (1974).

The MLR has been used to determine certain genotypes. So far, no recombinations between the serologically defined AgB-locus of rats and the locus determining reactivity in the MLR have been reported; therefore the MLR has been used to determine AgB-type. Moreover, the AgB-locus seems to be closely linked to a gene regulating susceptibility to EAE, designated the Ir-EAE-gene by Williams & Moore (1973, see also Gasser et al. 1973, 1975). Thus AgB-typing, with the aid of the MLR, will inform about the Ir-EAE-genes of hybrids in crossing trials between different inbred strains. However, the immunization for EAE has been reported to cause alterations in the MLR (I). Therefore typing for AgB-allele was performed before immunization in the present study (Appendix II).

Encephalitogenicity - Quantification

EP possesses different encephalitogenic sites (recently reviewed by Bergstrand 1977). In reports on encephalitogenicity, the evaluation technique is based on parameters such as incidence of diseased animals, onset of signs of EAE, and average clinical and histological severity (cf. Martenson et al. 1975). This is per se an adequate method for ranking different encephalitogenicities, as several parameters have been studied, but it does not permit any objective quantification. Small experimental groups often have been studied, and only one emulsion has been prepared for investigation of each antigen tested. In our opinion, presumably identical emulsions prepared on different occasions can display vastly different ac-

tivity. Paper IV showed that a significant source of variation exists in this respect. An example from the study on guinea pigs (III) is that in three groups of animals - injected on different occasions presumably with identical emulsions of EP in FCA - the incidence of histological signs (seven levels of CNS blind examined by two readers) was $4/4$, $2/4$, and $0/4$, respectively. We therefore stress the importance of using at least two, preferably three, emulsions of identical composition, processed on different occasions for test of encephalitogenic activity (cf. review by Kies 1973).

In order to be able to quantify the activity of an encephalitogenic site, we evolved a method that permits a quantification of the activity of, e.g., an encephalitogenic peptide in relation to an EP (II, III). These studies examine the encephalitogenic activity of different peptides of the bovine EP in relation to the intact EP in random-bred guinea pigs. These animals were studied with respect to incidence and mean score of histological signs, debut day and incidence of neurological signs, and weight loss amounting to more than 10% of weight recorded at the beginning of the loss. A dose-response effect was obtained with graded amounts of the bovine EP at immunization (III). The use of this made it possible to interpolate what activity a peptide of EP had in relation to the intact EP. Thus we could obtain a quantitative estimate on the effect of a peptide compared with intact EP, expressed in, for instance, per cent.

The histological examination of several levels of the CNS is an adequate procedure, because it will help to detect even minimum changes; that is to say, sometimes lesions were detected at only one level examined. A 0-2 score was used, and three changes of EAE were studied separately: meningitis, vascular cuffs, and infiltration. As seven levels were examined (blind), this produced a scale varying between 0 and 42 in the guinea pigs. As the separation into three histopathological changes of EAE gave no further information, a common score was used in the later studies on the rats, 0-3, which permitted a scale range between 0 and 21 for an animal. This method thus permits a more differential scoring of histological lesions than those usually used by previous workers, as they have had a common score for all levels, usually between 0 and 4 (cf. Martenson et al. 1975), but also sometimes more differentiated, 0-8 (cf. McFarlin et al. 1973). Our method has another strength in the blind examination of each level, as unbiased scoring cannot possibly be performed with knowledge of other parameters of the animal tested or of scores on other levels examined.

Concerning the different levels of CNS examined, it was confirmed how consistently the most severe lesions were found at the cerebellar level and how the most caudal level of the spinal cord seemed to be more affected than more cranial ones. This too should be seen in relation to the neurological

findings. It has been discussed whether serotoninergetic neurons, which are relatively frequent in the lumbar segments and are present in all CNS, should be especially involved in the pathogenesis of EAE. For instance, there is evidence for a reduction in such neurons at EAE (Lycke & Roos 1973).

Genetical Considerations

As genetical factors are of importance in the pathogenesis of MS, studies on such matters are of great interest at EAE. When Gasser et al. (1973) and Williams & Moore (1973) described the linkage of an Ir-EAE-gene to the AgB-locus of rat, this seemed to offer a simple model for study of inheritance of susceptibility to EAE. These reports concerned EAE in the highly susceptible Lewis rat and the very resistant BN rat and their hybrids in the F₁ generation and backcross of this generation with the parental strains. Susceptibility to EAE was interpreted as determined by a dominant gene (Ir-EAE) closely linked to the AgB-locus. On the other hand, Perlík & Zídek (1974), examining another resistant strain, found evidence for a dominant gene for resistance, and when the intermediate (with respect to EAE-susceptibility) DA rat was examined in such studies by Gasser et al. (1975), a much more complex mode of inheritance of susceptibility to EAE was apparent.

We studied the Lewis, PVG, and Fischer rat strains together with the F₁ (Lewis x PVG) hybrids and a backcross of this F₁ generation with the Lewis strain (IV, VI, Appendix II). Concerning combined criteria, the studies reveal an intermediate mode of inheritance of susceptibility to EAE when the Lewis and PVG rats are crossed. Backcross of this F₁ generation with the Lewis strain gave rats that tended to develop more severe neurological signs and less pronounced histological signs of EAE than would have been expected on the basis of an intermediate mode of inheritance. Thus, in Lewis backcross rats homozygous for the AgB-allele of Lewis, neurological signs were compatible with a dominant Ir-EAE-gene. However, the histological score was unexpectedly low. In the other Lewis backcross rats (thus with AgB-type of F₁ generation) the histological score was the expected (presuming close linkage of the gene regulating this parameter to the AgB-locus); but surprisingly, 50% (7/14) developed neurological signs. These signs are compatible with a balance between a dominant gene (Ir-EAE) closely linked to the AgB-locus and another postulated dominant gene of resistance on another chromosome. Fig. 1 shows how the genes are inherited. The resistance gene will partly suppress disease (neurological signs) when Ir-EAE-gene is heterozygous (LP) but not when it is homozygous (LL). This would explain why no neurological signs developed (except perhaps in one rat) among the F₁ hybrids, whereas 50% of the Lewis backcross rats with the same AgB-type (LP) did so. It

		Incidence of neurological signs:	
Generation	Genotype	Expected (%)	Found
Parent (Lewis, PVG)	LLrr PPRR	LL: 100	31/36
		PP: 0	0/10
F ₁ (Lewis x PVG)	LPRr	0	1*/11
Backcross of	LLRr	100	12/16
F ₁ (Lewis x PVG)	LLrr	100	
with Lewis	LPRr	0	7/14
	LPrR	100	

* questionable

Figure 1. Expected and found incidences of neurological signs of EAE in rats of different genotypes.

L=AgB-allele of Lewis, presumably closely linked to the Ir-EAE-gene

P=AgB-allele of PVG, presumably closely linked to the Ir-EAE-locus

R=postulated gene for resistance on another chromosome.

will explain too why the LL-homozygous Lewis backcross rats behaved like the parental Lewis strain with respect to neurological signs. The incidences of 12/16 and 31/36, respectively, in these groups are interpreted as not different.

The mode of action of such a resistance gene could then be the coding for the proliferation of a suppressor T-cell clone, but it can also affect B-cells, so that it induces, for instance, some kind of protective antibodies. There are other possible explanations.

Concerning the histological findings, no explanation is available. The study showed a strikingly less degree of correlation between clinical findings and histological signs than previously found (IV, VI). Waksman & Adams (1962) reported a "poor correlation of clinical and histological findings in the guinea pigs examined," so too did Robson et al. (1971), and Hughes & Stedronska (1973) and Allegretti & Marušić (1976) in studies on the rat, where clinical symptoms of EAE were reported in the absence of histological changes. Concerning the study by Hughes & Stedronska (1973), a very small experimental group can explain the findings as one obviously cannot examine every mm of the CNS histologically. In the study by Allegretti

& Marušić (1976), the absence of histological signs was reported in EAE-diseased rats that were T-cell deficient. On the other hand, the need for care in the interpretation of such findings has been emphasized by Levine et al. (1975).

The Fischer strains (VI) studied in our laboratory was, with combined criteria, intermediate between the F₁ (Lewis x PVG) and PVG rats. However, there was a tendency to dissociation between histological findings and a clinical symptom: weight loss. For a given degree of clinical state (i: healthy, ii: weight loss less than 10%, iii: weight loss more than 10%), the Fischer rats seemed to display less histological lesions than the rats previously examined. In this context, however, it must be questioned whether or not weight loss is a relevant criterion for EAE. Rats immunized with saline and FCA (VI) did not show any weight loss; it is therefore obviously not a consequence of the FCA injection, but probably reflects a true effect obtained with the encephalitogenic antigen. Probably, the susceptibility to EAE in animals is the result of a balance between different genes, and this might have relevance for the pathogenesis of MS.

Cell-Mediated Immunity

As mentioned in the Introduction, the significance of autoimmune phenomena in the aetiology and/or pathogenesis of MS has been much debated. Naturally, special interest has been paid to cell-mediated immunity to EP and peptides derived from it. Various techniques have been employed to demonstrate such immunity. During recent years, a series of studies from our laboratory have been published using the leukocyte migration in agarose technique. These studies have shown that a reactivity to EP can be demonstrated at MS and at other diseases involving CNS destruction. A weak reactivity can be demonstrated in some healthy subjects.

Some studies suggested that the reactivity to EP is correlated with the disease type of MS: patients with a chronic progression showed the strongest reactivity in the tests (Berg et al. 1975). This study indicated a special significance of the tryptophan-containing determinant of EP for the outcome of the test at MS. Further studies (Källén et al. 1977a,b) indicated that there was a marked change in reactivity in connexion with a relapse of MS and (Nilsson & Thelin 1977) that a correlation exists between the outcome of the test and parameters indicating intrathecal synthesis of immunoglobulin with a disturbed kappa:lambda chain ratio in CSF. However, the picture is complicated by the fact that, in connexion with a cerebral infarction, dramatic changes in leukocyte migration reactivity can also occur (Källén et al. 1977b). Some patients reacted strongly immediately after a stroke - this could be an immuno-

logical secondary response - others showed no reactivity until some weeks after the stroke: this could possibly represent a primary response.

Undoubtedly, in vitro reactivity to EP can be seen both at MS and at non-MS conditions. It is not yet determined whether the reactivity described at MS in some way differs from that seen after other CNS destruction. The latter is obviously a secondary consequence to the tissue destruction and sensitization to released antigens from CNS.

At EAE, sensitization of the animal is brought about experimentally, and the cell-mediated reaction, registered in various in vitro tests, is obviously a consequence of the immunization procedure. It can be demonstrated already before the onset of the disease. Bergstrand & Källén (1971) even demonstrated that transformation of rabbit peripheral blood leukocytes by EP occurred mainly before the onset of clinical signs and could disappear at the peak of the illness. Vandembark & Hinrichs (1974) studied rat peritoneal exudate cells and presented data indicating that reactivity (measured by migration inhibition technique) was present before, during, and after illness, but reached a peak during illness. In the present studies (V, VI), lymph node cells were used some time after the disease period (day 28), and secondary responses to the break-down of CNS tissue can have occurred; but as regional nodes to the immunization site were used, the major part of the reactivity is reasonably a direct consequence of the immunization.

In vitro reactivity to EP might not, for various reasons, correlate with EAE disease. EP or parts of it that are not encephalitogenic in one species or strain might, none the less, cause a cell-mediated immunological response, demonstrable in the in vitro test, and could even cause an equally strong in vitro reaction as an encephalitogenic antigen does. In the rat, for instance, bovine EP is not, or only weakly, encephalitogenic, but the in vitro reactions obtained are of the same magnitude as those obtained with highly encephalitogenic EPs (Vandembark & Hinrichs 1974, V). In the guinea pig, peptide 1-42 of the bovine EP is only weakly encephalitogenic, if at all (II, III), yet it is as good a stimulator of cell-mediated immune reactions - studied with the macrophage migration inhibition technique - as more encephalitogenic parts of the EP molecule (Bergstrand & Källén 1973). In the rat, it was not possible to show that the "major encephalitogenic site" of guinea pig EP gave a greater in vitro reaction than the weakly encephalitogenic parts of the molecule did (V, VI). Evidence for a dissociation between in vitro cell-mediated reactivity and encephalitogenicity has also been obtained by Spitler et al. (1972, 1975).

Thus it is understandable that rats with different genotypes and different susceptibility to EAE might show very similar in vitro responses to EP (V, VI). This differs slightly from

findings previously made with the more resistant BN rats (McFarlin et al. 1975a,b) where almost no in vitro reactivity to EP was found. It is possible that this strain lacks Ir-genes and is therefore unable to mount a cell-mediated response against any part of EP.

One possible explanation to the dissociation between susceptibility to EAE and the in vitro cell-mediated response to EP (V, VI) is that, although the rats of different genotypes can all react to non-encephalitogenic parts of EP, the difference in susceptibility can be due to reactivity to the small amino acid sequence that makes up the major encephalitogenic site of the molecule. Our studies using peptides derived from guinea pig EP do not support this idea but cannot rule it out, as the peptide studied is larger than the actual determinant and might contain non-encephalitogenic determinants that can give the in vitro reaction. It is even possible that differences between strains are due to different reactivity to suppressive sites on EP (Swanborg 1975) - the reactivity in rats with no, or only slight, disease might mirror the response to such sites. In guinea pigs, suppression of EAE can be obtained if the animals are (pre)treated with aqueous brain suspensions, EP, encephalitogenic or non-encephalitogenic peptides, or even with peptides of random sequence in Freund's incomplete adjuvant (without mycobacteria) (Rauch & Einstein 1974, Swanborg 1975, Driscoll et al. 1976). On the other hand omission of the mycobacteria does not seem to induce suppression in the Lewis rat, as Levine & Wenk (1965) reported that a saline homogenate of homologous CNS tissue (without adjuvants) induced EAE in this strain. This, of course, does not argue against the possibility that FCA+EP can stimulate suppressor cell clones in the rat. Driscoll et al. (1976) reported (unpublished observations) that the pretreatment of (probably) guinea pigs with FCA completely suppressed EAE. Contrary to this, we have seen histological lesions of EAE after FCA immunization in this species (II, III). These phenomena should also be seen in connexion with the weak cross-reactivity between FCA and EP (Bergstrand & Källén 1973, McDermott et al. 1974, Vandenbark et al. 1975). Thus the problem of suppression seems rather complicated.

Another possibility is that the high and low susceptibility strains mount the same cell-mediated immune response to EP, but the difference in disease development is due to other differences, e.g., antigen presentation in the target organ, development of protective antibodies, etc.

Finally, perhaps the different strains do in fact show different strength in immunological response, but the in vitro techniques cannot differentiate between them. For instance, it is possible that tests with lower antigen concentrations would have distinguished between the different response levels, but the weak stimulations seen even with the high antigen amounts do not encourage studies with lower concentrations.

SUMMARY

The mixed leukocyte reaction (MLR) was studied between cells from rabbits with experimental allergic encephalomyelitis (EAE). Challenge for EAE gave a reduction in MLR due to an effect on both the stimulator and responder phase. MLR was also depressed between cells from EAE rabbits and healthy control rabbits. Similar but weaker effects were seen after immunization with Freund's complete adjuvant (FCA). Sera from EAE rabbits depressed the MLR between cells from healthy rabbits. An attempt to isolate the factor(s) responsible showed that these were very labile and difficult to characterize.

Bovine encephalitogenic protein (EP) was examined with respect to encephalitogenic sites in guinea pigs and a quantification method for encephalitogenicity was presented. If active at all, peptide 1-42 has an encephalitogenicity of only approx. 1% of the intact EP; corresponding value for peptide 43-115, tyr (the tyrosine residue is oxidized by the preparation procedure) is 7%, for peptide 89-169 100%, and the latter peptide with the tryptophan residue (residue 115) blocked by 2-hydroxy-5-nitrobenzylation displayed 15% of intact EP activity. A dose-response effect with graded amounts of bovine EP at immunization was demonstrable.

Inbred rats of the Lewis, PVG, and Fischer strains, and the F₁ (Lewis x PVG) generation and a backcross of this F₁ generation with Lewis were studied. The high susceptibility to EAE of the Lewis strain was confirmed. The PVG strain was rather resistant, and the F₁ generation was intermediate. The Fischer strain was intermediate with respect to the F₁ (Lewis x PVG) and PVG rats. The Lewis backcross rats displayed a higher incidence and severity of neurological signs and lower severity of histological signs than would have been expected. The studies on the Fischer rat also indicated a possible existence of a dissociation between clinical and histological signs. Guinea pig EP was highly encephalitogenic compared with the weakly active bovine EP. A positive correlation between mycobacterium content of FCA and disease was shown.

Despite the above findings, rats of the various genotypes examined could mount equal in vitro cell-mediated immune responses. This was not affected by the amount of mycobacterium content, and bovine EP was as effective as guinea pig EP. Guinea pig EP sensitization causes a partial crossreactivity to bovine EP and the reciprocal crossreactivity also probably exists (in rabbits it was more marked). The peptide encompassing the major encephalitogenic site for rats could not be shown to react stronger in vitro with cells sensitized to the intact guinea pig EP than other peptides tested.

ACKNOWLEDGEMENTS

The costs of this investigation were defrayed by grants from the Swedish Medical Research Council (Project B 75-16X-3920) and funds of Medical Faculty, Lund, Sweden.

My sincere thanks to the colleagues and the technical staff of the Tornblad Institute and the technical staff of the Department of Anatomy for help and technical assistance; the skilful assistance of Mrs Christina Boll, Miss Karin Hansson, Mrs Ingegerd Andersson, and Mrs Elsie Nilsson is especially appreciated.

The English text was revised by Mr W.F. Salisbury.

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APPENDIX I

Studies on Serum Factor(s) Responsible for the Depression of Mixed Leukocyte Reaction at Experimental Allergic Encephalomyelitis

SUMMARY

An attempt was made to isolate and characterize, from sera from rabbits with experimental allergic encephalomyelitis, the factor(s) responsible for the suppression of the mixed leukocyte reaction between healthy rabbits (Lindh 1973). The factor is not dialysable but relatively labile and could not be allocated to any specific fraction at separation on a Sephadex G-200 column.

INTRODUCTION

A previous report (Lindh 1973) showed that sera from rabbits with experimental allergic encephalomyelitis (EAE) could suppress the mixed leukocyte reaction (MLR) between cells from healthy rabbits. A similar suppressive effect of serum on in vitro lymphocyte reactions has been described in EAE (Vandenbark & Burger 1974, Vandenbark et al. 1974) and in multiple sclerosis (MS) (Knowles et al. 1968, Stjernholm et al. 1970, Field & Caspary 1971, van den Noort & Stjernholm 1971, White et al. 1975). We have tried to further characterize the factor(s) responsible for the MLR inhibiting effect.

MATERIAL AND METHODS

14 rabbits were immunized in three groups of four or five on different occasions with bovine encephalitogenic protein (EP), 2mg/ml (saline solution) emulsified in an equal amount of Freund's complete adjuvant (Difco). Fresh emulsions were prepared each time, and 0.05ml was injected in each of three toe pads. Sera were obtained before immunization and on different occasions after immunization, were heat-inactivated at 56°C for 30 minutes, and tested in the MLR between healthy rabbits.

Three pairs of sera (one before and one after immunization) with suppressive activity were selected for fractionation on a Sephadex G-200 column. This was done twice with each pair of sera. Four major fractions were obtained, three corresponded to the three major peaks obtained, the fourth constituted material eluting after the third major peak. The various fractions are presumed to contain, among other components, the following:

- I: alpha₂-macroglobulins, beta₂-lipoproteins, IgM
- II: IgG, IgA, IgE
- III: albumin, lymphokines
- IV: possible lymphokines (of minor size)

The fractions were concentrated to between half or one third of the volume of the original whole serum (WS), i.e. molar concentration of constituents was two to three times that of WS. The medium used at MLR with test of WS contained 33% WS and 67% Parker 199 + antibiotics and heparin. When fractions were tested, the medium contained fraction constituents in an amount equimolar to that in which they appeared when corresponding WS was used in the medium. Furthermore, the medium contained 20% normal rabbit serum (for supply of essentials to the leukocytes) and Parker 199, adjusted so that the concentration of salts etc. would correspond to the medium with WS. For instance, if a fraction was concentrated to half the volume of the original WS, the medium was composed of 16.5% fraction, 20% normal rabbit serum, and 63.5% adjusted (concentrated) Parker 199, so that this would correspond to 67% normal Parker 199 (thus analogous to when WS was tested). Antibiotics and heparin were added as above, and finally, these media (composed with fractions) were filtered through a millipore filter (0.22µm Ø, Millex, Millipore Corporation, Bedford, Massachusetts). (For further preparation of the MLR, see Lindh 1973.) After seven days of incubation at 37°C, the uptake of added ³H-thymidine was assayed as described by Lindh (1977). The suppressive activity of a serum or fraction after immunization was calculated as the difference between log₁₀ (c.p.20min.) ³H-thymidine uptake in cultures with serum or fraction before immunization.

Dialysis was performed in a 2cm Ø tube (cellulose, with a porediameter of 24Å, carefully washed in distilled water and

sterilized) for 24 hours at 4°C. Before dialysis, culture medium was prepared of the sera that were selected for dialysis, as described above; after dialysis, these media were filtered through a millipore filter (0.22µm Ø).

RESULTS

Significantly suppressive activity ($p < 0.05$) has been traced to all four fractions, but it was usually present in only one or two fractions at an individual testing. (A total of 13 such tests were performed.) In one test, activity was seen in three fractions, but never in all four fractions at one testing occasion. Repeated tests of a pair of WS and fractions revealed that the activity could change between fractions. The same inconsistency of activity occurred between different pairs of WS and fractions, but also between different fractionations of a certain pair of WS. Table 1 shows the results from two testing occasions. Significantly inhibitory effect was seen in WS and fractions I, II, and IV, whereas a test of another pair of WS and its fractions revealed significantly inhibitory effect in WS and fractions III and IV.

The sera were stored at either -20°C (Figure 1A) or -80°C (Figure 1B). A time study on suppressive activity showed a tendency for it to increase during storage, reach a peak, and later gradually disappear. Suppressive activity was absent after 10-12 months of storage. This change in suppressive activity showed a tendency to run a somewhat slower course when sera were stored at -80°C instead of -20°C.

In order to study whether thawings and freezings could affect activity, one serum was selected for such a test. A gradual in-

Table 1. Inhibitory(-)/stimulatory(+) effects recorded in the MLR with two different sera and their fractions as a result of immunization for EAE. The values express the difference between uptake of ³H-thymidine ($\log_{10}$ c.p.20min.) in MLRs with after-immunization and before-immunization serum (fraction).

Serum or fraction tested	Serum pair 1	p	Serum pair 2	p
WS	-0.53	<0.001	-0.58	<0.05
I	-0.27	<0.05	+0.16	NS
II	-0.24	<0.05	+0.44	NS
III	-0.03	NS	-0.61	<0.05
IV	-0.62	<0.001	-0.62	<0.05

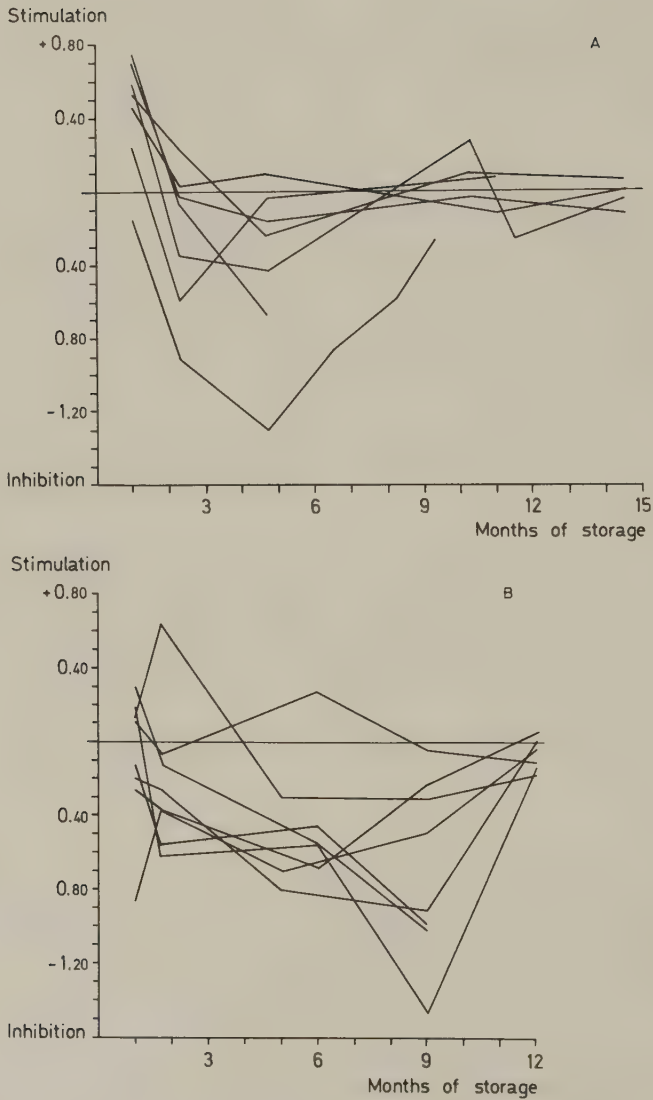


Figure 1. Inhibition of MLR calculated as difference between ^3H -thymidine uptake ($\log_{10}\text{c.p.20min.}$) in cultures with serum after immunization and before immunization for EAE. The change in this difference during approx. one year in freezer at -20°C (A) or -80°C (B) is illustrated.

crease in suppressive activity of the "after-immunization" serum occurred as a result of eight consecutive thawings and freezings, whereas "pre-immune" serum was not markedly affected (Figure 2). Regression analysis showed that this effect was significant ($p < 0.02$).

One "after-immunization" serum was dialyzed against its "pre-immune" serum. Controls revealed that no change occurred in either "pre-immunization" or "after-immunization" activity (Figure 3) and that a mixture of these sera (not dialyzed) gave an intermediate activity.

DISCUSSION

This study shows and confirms the difficulty in tracing and isolating the immunosuppressive activity of a serum, as discussed by Cooperband (1974). The time-course study and the experiment with thawings and freezings indicate the rather

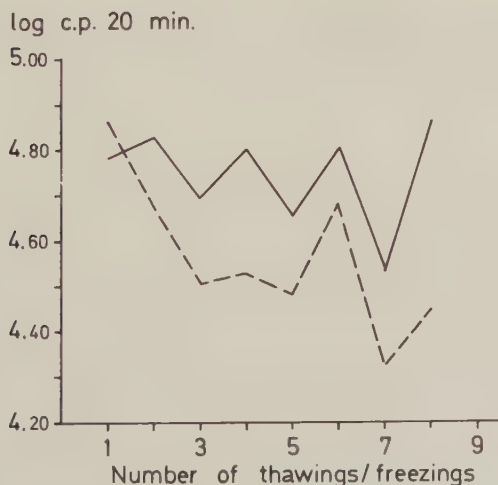


Figure 2. The effect of eight consecutive thawings and freezings on a pair of sera. Before immunization:—, After immunization:----. Values of ^3H -thymidine uptake ($\log_{10}$ c.p. 20 min.) are given.

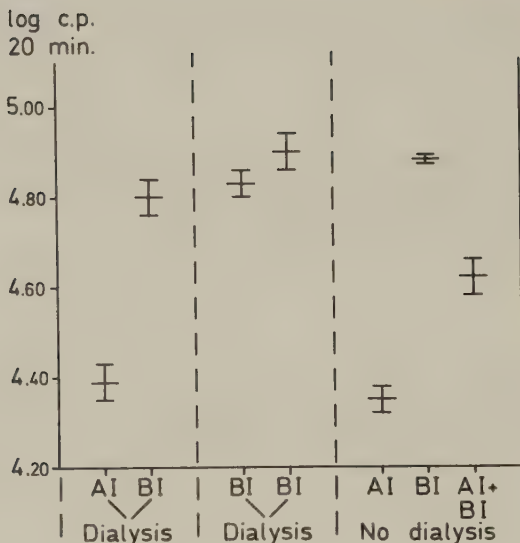


Figure 3. Effect of dialysis of after-immunization serum (AI) against before-immunization serum (BI) and of mixture of the two. Mean of uptake of ^3H -thymidine ($\log_{10}\text{c.p.20min.}$) is given with standard error for the mean of each three tubes.

labile nature of the active factor(s). Similar labile character was reported previously also for the lymphotoxic activity of MS-serum (Stjernholm et al. 1970, White et al. 1975).

A balance perhaps exists between factors that can either stimulate or inhibit the immune response. The significance of the inhibiting factors should then be to suppress the immunological attack on the CNS at EAE. This hypothesis can have relevance also for the weak immunosuppressive effect of sera at MS on the MLR (Hedberg et al. 1973) and was suggested for the lymphocyte response depressive factor in MS by Field & Caspary (1971) and White et al. (1975). However, the very labile character of the activity registered in this study makes it unlikely that we are dealing with a "protective" antibody similar to that previously discussed by Paterson et al. (1965).

Activity could be traced to different fractions in different sera. This can be due to the factors being loosely bound to their normal carriers; and because of the processing conditions, a random binding happens to other carriers. But as this vari-

ation occurred within a certain pair of WS and fractions, it indicates the presence of other phenomena. To judge from the dialysis experiment, no activity can be ascribed to minor molecules not bound to carriers. This non-dialyzable character resembles that of the lymphotoxic factor from MS-serum, described by White et al. (1975).

ACKNOWLEDGEMENTS

My sincere thanks to Dr. H. Bergstrand, who fractionated the sera and also supplied the bovine EP.

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APPENDIX II

The Susceptibility to Experimental Allergic Encephalomyelitis
of a Backcross of F_1 (Lewis x PVG) and Lewis Rats

SUMMARY

A previous paper (Lindh 1977a) on the susceptibility to experimental allergic encephalomyelitis (EAE) in Lewis and PVG rats and their F_1 hybrids has been extended with a study on the generation obtained by backcross of the F_1 hybrids with the susceptible parental Lewis strain. These Lewis backcross rats tend to develop more severe neurological signs and less pronounced histological signs of EAE than would be expected on the basis of an intermediate mode of inheritance. The findings are discussed in the light of the presumed close linkage between the AgB locus and the Ir-EAE gene and the possibility of another postulated resistance gene on another chromosome.

INTRODUCTION

The Lewis rat is very susceptible to experimental allergic encephalomyelitis (EAE). A previous paper (Lindh 1977a) studied this strain together with the rather resistant strain PVG and their F₁ (Lewis x PVG) hybrids. It was stated that, with combined criteria, the F₁ hybrid generation was intermediate in EAE susceptibility compared with the parental strains, a finding similar to that published by Gasser et al. (1975). The immunization procedure and the criteria used for EAE evaluation will influence whether susceptibility could be considered dominant or recessive in character (Lindh 1977a).

This paper presents a study on the generation obtained by backcross of the F₁ (Lewis x PVG) generation with the highly EAE-susceptible Lewis parental strain. EAE has been evaluated by clinical and histological criteria, estimating both incidence and strength. It has been hypothesized that there exists a gene, called Ir-EAE, which regulates susceptibility to EAE and is closely linked to the major histocompatibility locus in the rat (Gasser et al. 1973; Williams & Moore 1973). The Lewis backcross rats were therefore divided into two groups according to AgB type, using the one-way mixed leukocyte reaction (MLR) test, as no recombinations between the serologically defined AgB locus and the locus determining MLR have yet been reported.

MATERIAL AND METHODS

Rats. The Lewis backcross rats were obtained by crossing and backcrossing inbred Lewis and PVG rats (obtained from Møllegaards Avlscentrum, Ejby, Denmark). On three different occasions, 33 Lewis backcross rats were immunized (without knowledge of AgB-type) at age 2.5-3.5 months with freshly prepared emulsions. Food and water was provided ad lib. During the 28 days post immunization, the animals were weighed daily and assessed for neurological signs, as described previously (Lindh 1977a).

Immunization. The animals were injected in both hind paws with guinea pig basic protein of myelin (encephalitogenic protein or EP) in a saline solution emulsified with an equal volume of Freund's complete adjuvant; 0.05ml of the emulsion in one toe pad of each hind paw. Each rat received 50µg of the guinea pig EP and 125µg of Mycobacterium butyricum. (For corresponding preparation of the bovine EP, see Bergstrand 1971.) The guinea pig EP was kindly supplied by Dr. H. Bergstrand.

MLR cultures for AgB typing. This was performed some weeks before immunization. A piece of the spleen was excised for further

preparation in the MLR test. The Lewis backcross cells were stimulated (one-way MLR) with "typing" cells: Lewis cells or F_1 (Lewis x PVG) cells; if AgB type is Lewis/Lewis, AgB(L/L), cells will respond to F_1 (Lewis x PVG) cells but not to Lewis cells. If AgB type is Lewis/PVG, AgB(L/P), none of the "typing" cells will cause a stimulation. The excised spleen pieces were trimmed and washed with Parker 199, and a cell suspension was prepared and filtered through gauze. Cultures were set up with 2×10^6 cells from each cell donor in 1.5ml of a medium consisting of 20% rat serum (heat-inactivated at 56°C for 30 minutes) in Parker 199 with antibiotics and heparin. The one-way MLR was obtained by incubation of the "typing" spleen cells with mitomycin C (Sigma), $25\mu\text{g/ml}$ for 20 minutes at 37°C , followed by repeated washing with Parker 199. Cultures were set up in triplets and were incubated at 37°C for five days. The uptake of ^3H -methyl-thymidine was then assayed as described previously (Lindh 1977b) and expressed as $\log_{10}$ of the sum of the c.p.m. during two 10-minute periods.

Histological lesions. Seven levels of the central nervous system were taken for histological preparation, as described previously (Lindh 1977a), and were scored blind with a 0-3 score for each section, 0 indicating no EAE lesion (for further details, see Lindh 1977a).

RESULTS

AgB typing. Recorded ^3H -thymidine incorporation in triplet tubes can give an estimate of the technical variation in the MLR system. It could be determined to 0.0456 at 100 d.f. This implies that the difference between the means of two triplets has a variance of 0.0304. Only once in 100 experiments should a difference of 0.48 be reached randomly. When the incorporation obtained after stimulation with AgB(L/L) cells is compared with that seen after stimulation with AgB(L/P) cells, a difference of 0.5 or more can hardly be random, but indicates that the tested rat is AgB(L/L): a difference less than 0.4, on the other hand, is probably random and the rat is AgB(L/P). With these criteria, 16 of the Lewis backcross rats were classified as AgB(L/L) (range: 0.50 to 1.01; mean: 0.69) and 14 were AgB(L/P) (range: -0.33 to 0.34; mean: 0.18); the remaining three showed differences between 0.4 and 0.5 and therefore could not be typed (unclassifiable).

Neurological signs. Of the 16 AgB(L/L) backcross rats, 12 developed neurological signs of EAE, 10 even had total hind leg pareses, see Table 1. Of the 14 AgB(L/P) backcross rats, 7 developed neurological signs, 6 of them had total hind leg pareses, and of the 6, one died on day 16 after immunization. Of the unclassifiable rats, one was apparently healthy, one displayed weakness, and one had pareses.

Table 1. Distribution of rats in backcross of F₁ (Lewis x PVG) with Lewis rats with respect to MLC determination of AgB type with the predictions made in "Introduction". Frequencies of neurological signs of EAE are given.

AgB-type of Lewis back- cross rat	without neuro- logical signs	weakness	total pareses	Total
L/L	4	2	10	16
L/P	7	1	6	14
unclassifiable	1	1	1	3

Total	12	4	17	33

The debut day of neurological signs was from day 10 to 15 after immunization. The arithmetical mean for the AgB(L/L) group was 12.3, and for the AgB(L/P) group, 12.7.

Weight loss. Almost all animals lost weight, usually more than 10% of weight before onset of loss. No difference could be noted between the two groups of Lewis backcross rats.

Histological lesions. Figure 1A shows the histological score (sum of seven levels) for individual backcross rats of AgB(L/L) type. The median is 6.0 with range 0-13. Of the rats with total hind leg pareses (degree V), the histological score was significantly lower for Lewis backcross rats than for the parental Lewis rats (Lindh 1977a) ($p < 0.01$; Wilcoxon's rank test), and the same significance level was also reached when rats of degree IV and V were pooled together.

In the AgB(L/P) group (Figure 1B), the median is 4.5 with range 0-11. In both groups, rats without neurological signs appear to have histological EAE to a greater extent than, or as severe as, rats with neurological signs.

The incidence of histological changes in AgB(L/L) group was 15/16, and in AgB(L/P) group, 11/14.

DISCUSSION

A previous study (Lindh 1977a) showed that neurological signs of EAE developed in most (31/36) Lewis rats immunized according to the schedule used in the present study. This agrees well with the incidence found among the Lewis backcross rats which

here, more F_1 hybrids than the backcross rats (to both parental strains) with the same AgB-type tended to develop clinical signs. Whereas the BN rat is considered a rather resistant strain and is almost incapable of mounting an in vitro cell-mediated immune response to sensitizing antigen (McFarlin et al. 1975), the PVG rat is a low responder strain, which mounts an in vitro cell-mediated immune response to sensitizing antigen as good as does the highly susceptible Lewis rat (Lindh 1977b). Thus genetic differences seem to exist between the BN and PVG rats in their regulation of the response to immunization for EAE.

In order to explain the findings in this Lewis backcross generation, a more complicated mode of inheritance of susceptibility to EAE must be postulated. One such possibility is the presence of EAE susceptibility genes linked to the AgB locus (Ir-genes), and resistance genes, possibly on another chromosome. A susceptibility gene (S) is apparently carried by the Lewis strain linked with the AgB-L gene; the PVG strain could then carry a resistance gene (R) not linked to AgB-P. The F_1 hybrids will have the genotype SsRr: they do not develop neurological signs, and thus the R gene can counter-balance one S gene. Half of the Lewis backcross rats will be Rr, half will be rr. In the AgB(L/L) backcross rats, the presence of the R gene apparently plays no significant role, as the expected rate of neurological signs is realized. The susceptibility gene, when present in a homozygotic state, can apparently overcome a heterozygotic resistance gene. In the AgB(L/P) backcross rats, however, the Rr alternative will suppress neurological signs of EAE, but the rr alternative will permit the penetration of the heterozygotic susceptibility gene. This two-gene hypothesis would thus explain the found inheritance of susceptibility to EAE with respect to neurological signs.

The histological findings of EAE gave a somewhat unexpected distribution, especially when related to the neurological signs. Among the Lewis backcross rats that were AgB(L/P), nearly the same incidence and score of histological signs were found as in the previously reported F_1 rats (Lindh 1977a). Almost all animals showed some histological lesions, and the median score agreed relatively well between the two groups: F_1 rats - 6.5 and Lewis backcross rats, AgB(L/P) - 4.5. Thus the marked difference in neurological signs between these two groups of rats (with identical AgB genotype) does not correspond to a difference in histological score.

Among the Lewis backcrosses with AgB(L/L) genotype, the histological score and the incidence of histological changes were very similar to those found among the Lewis backcross rats with AgB(L/P) genotype, but were markedly lower than those previously found in the parental Lewis strain: they had a median score of six, whereas the parental Lewis rats had 12. Thus the Lewis backcross rats with AgB(L/L) genotype had as many neurological signs as the parental Lewis rats, but only half as severe histological lesions ($p < 0.01$).

The present study found the individual correlation between neurological disease and histological changes to be much less pronounced than did the study on the parental strains and the F₁ hybrids (Lindh 1977a). This is apparent from Figure 1, which shows that many rats with slight or no clinical signs of EAE had marked histological changes, and vice versa. A segregation of neurological signs and histological changes thus occurs according to the genetic background of the animals. Some previous studies indicate that these two parameters can be dissociated by changing the immunological state of the rat. Alleghretti & Marušić (1976) reported that of 16 immunologically intact WVM rats (derived from Wistar stock) challenged with rat brain homogenate in FCA, 6 developed total paralysis and showed histological signs with mononuclear cell infiltrations and extravasates (frequency not reported). Of 37 T cell depleted WVM rats, 20 showed total paralysis, but no cellular infiltrations were detected. So high a frequency of diseased animals without cellular infiltration has never previously been reported. But it should be noted that under special experimental conditions (cf. Levine & Hoenig 1971, Levine & Sowinski 1971, 1972) the usual mononuclear cell infiltration can be absent or at least strongly reduced; it is then replaced with an infiltration of other cells (neutrophils).

The question whether or not a dissociation can exist between neurological signs and histological changes at EAE remains open (cf. Levine et al. 1975). It seems possible under special immunological conditions and, according to the present study, at certain genotypes. The actual mechanisms involved are so far unclear.

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Lund 1977